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Interactions of Indoleacetic Acid and Gibberellic Acid in Leaf Abscission Control

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Abstract. **Debladed midribs of citrus leaves showed the typical delay of abscission in response to indoleacetic acid (IAA), and the typical acceleration of abscission in response to gibberellic acid (GA). Interaction experiments with these 2 hormones indicated that the balance of the 2 hormones may be more important in regulating abscission than the quantity of either. The often reported acceleration of abscission with low quantities of IAA did not seem to exist in citrus. IAA did accelerate abscission in this tissue when its application was delayed for at least 24 hours after deblading, which suggests the 2-stage effect is also present in citrus.**

When abscission was first delayed with IAA and then allowed to continue, the rate of abscission proceeded at a slower rate than was typical for this tissue. This slower rate was also typical of the effect observed when GA overcame the abscission retarding effect of IAA. The phenylurethane, Barban, blocked the GA acceleration of abscission, but it did not affect the rate of abscission of control or IAA treated midribs.

Auxins and gibberellins have been shown to be involved in the control of leaf abscission of many plants (2, 6, 10, 11, 13, 18), including citrus (14); however, little has been done with the interactions of these 2 hormones. Thereafter, it was the goal of this research to study such interactions. It was also intended to compare a more nearly intact plant system with an explant bioassay, since the ultimate test of our knowledge of abscission must involve an intact plant.

Materials and Methods

Leaf Explant Bioassay. The explants used in the experiments were prepared from fully developed leaves of Valencia Late variety of sweet orange (*Citrus sinensis,* Osbeck). The leaves were cleaned with 10 % chlorox (sodium hypochlorite) above and below the abscission zone to avoid fungal and bacterial infections. Each explant was 13 mm long, including 10 mm of petiole and 3 mm of leaf midrib (fig 1B).

Explants were mounted with their proximal ends inserted into 4 % (w/v) sterilized agar, in a 100 × 20 mm petri dish as shown in figure 1C. The substances to be tested were dissolved in 0.1 M citrate buffer with pH 6.0 and the solutions were incorporated into equal volume of 2 % (w/v) agar. A 5 μliter droplet of the agar gel, containing the test compound, was applied at the distal end of each explant with a tuberculin syringe. The control explants were treated with a similar droplet of plain 1 % (w/v) agar.

Treated explants were placed in an incubator in the dark at 25°. Abscission was tested by applying a pressure of 5 g to the distal end of the midrib, with a calibrated wire spring mounted on forceps (1). The IAA was purchased from Sigma Chemical Company and the gibberellic acid (GA) was supplied by Merck Chemical Company.

Debladed Midrib Bioassay. Potted seedlings of sweet orange about 9 months old were selected for maximum uniformity of height, leaf color, and leaf quality. The growing terminal was removed, leaving only apparently mature leaves. Leaf blade and petiolar wings were removed, leaving a 4 mm portion of the leaf midrib and the petiole (fig 1D). Preliminary experiments showed no correlation between the position of the debladed midrib on the seedling and the order in which they abscised. The compounds to be tested were applied at the distal end of the midrib in a manner similar to that of the leaf explants. Treated seedlings were retained in the growth chamber in the dark at 25°.

In experiments where hormonal treatments were to be applied at different time intervals after leaf removal, the length of the midrib was increased to 6 mm, of which 2 mm were removed just prior to application of compounds to facilitate uniform uptake through a freshly-cut surface.

Statistical Methods. The statistical evaluation of treatment effects was based on time taken for 50 % abscission. The value for the time for 50 %

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abscission was calculated by fitting the data to a straight line equation of log y = log a + X log b which was derived from the function $y = ab^x$. Keeping log $y = y$, log $a = a$, and log $b = b$, the value of b was calculated as follows:

$$b = \frac{\Sigma \times Y - \Sigma \times \Sigma\ Y/n}{\Sigma x^2 - (\Sigma x)^{2}/^{n}}$$

Taking a as log of 0.5, *i.e.*, 50 % abscission and keeping Y as the log mean of the proportion of explants not abscised, the value of X for the time taken to reach 50 % abscission was calculated by the equation:

$$a = Y - bX$$

The coefficient of correlation for the best fit of the line was examined and the values obtained showed a good fit. The analysis of variance was worked out on factorial design for the 50 % values. The LSD or the shortest significant range values were calculated as necessary. Functional analysis of variance was done to assess and sort out specific treatment or interaction effects as the data warranted.

Results

A Comparison of the Explant and the Debladed Midrib Bioassays. The actions of IAA and GA on abscission of the citrus leaf explant were similar to those observed for other plant materials. Concentrations of IAA (table I) higher than 0.1 μg per explant retarded abscission. The lower concentrations, 10^{-4} and 10^{-5} μg, tended to cause the stimulation of abscission seen by others (3, 4, 8), but the effect was never statistically significant.

Concentrations of GA from 10^{-5} to 10 μg per explant significantly accelerated abscission (table I); however, the time to 50 % abscission was correlated with concentration only up to 10^{-3} μg. From 10^{-3} to 1 μg, there was no decrease in the time to 50 % abscission and 10 μg decreased the time to 50 % abscission compared with 1 μg. The decreased effectiveness of 10 μg of GA compared with lower concentrations was observed consistently in numerous experiments.

In all the above experiments, the hormone was applied immediately after deblading. Since Rubenstein and Leopold (17) and Chatterjee and Leopold (6) have demonstrated that IAA accelerates abscission of bean petioles if applied later than 9 hours after deblading, a similar experiment was done with the citrus explants. No acceleration of abscission by delayed applications of IAA was observed; applications as late as 36 hours after deblading still retarded abscission (fig 2).

Table I. *Response of Citrus Leaf Explants to IAA and GA*

Times to 50 % abscission were calculated by fitting the data to the straight line equation. Observations were made every 16 hours up to 128 hours. At the end of the experiment 0 %, 2 %, and 18 % of 10, 1, and 10^{-1} μg treatments, respectively, had abscised.

Hormone per explant	Time to 50 % abscission IAA	GA
μg	*hrs*	*hrs*
10	>303	79
1	>303	63
10^{-1}	303	66
10^{-2}	113	67
10^{-3}	109	68
10^{-4}	97	75
10^{-5}	96	86
0	101	96
LSD - 5 %	35	5

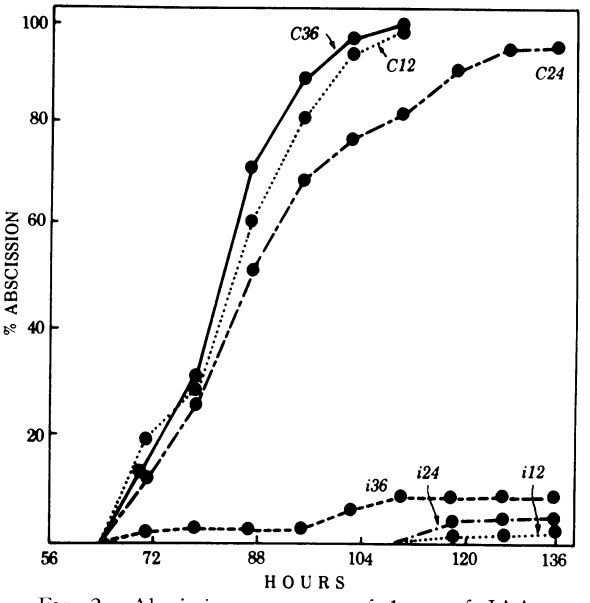

FIG. 2. Abscission responses of 1 μg of IAA per explant, applied at different time intervals after preparation. The treatments i-12, 24, and 36 indicate the hours after explant preparation when IAA was applied. Treatments C12, C24, and C36 are the respective controls. Fresh plain agar was applied at the corresponding time to controls.

Just as with the explant, higher levels of IAA retarded and all levels of GA accelerated abscission of the debladed midrib (table II). The GA effect did not increase proportionately with the concentration of the hormone in this system either, and compared with the large effect of 1 μg of IAA, was a relatively small effect. The low level of auxin again tended to accelerate abscission but the effect was not significant. When the auxin was removed by cutting off the end of the midrib, the first midribs abscised about 4 days later. Abscission then continued to completion; however, the rate of abscission was slower so that from the time of IAA removal to 50 % abscission was about 8 days rather than the 5 days typical of the freshly

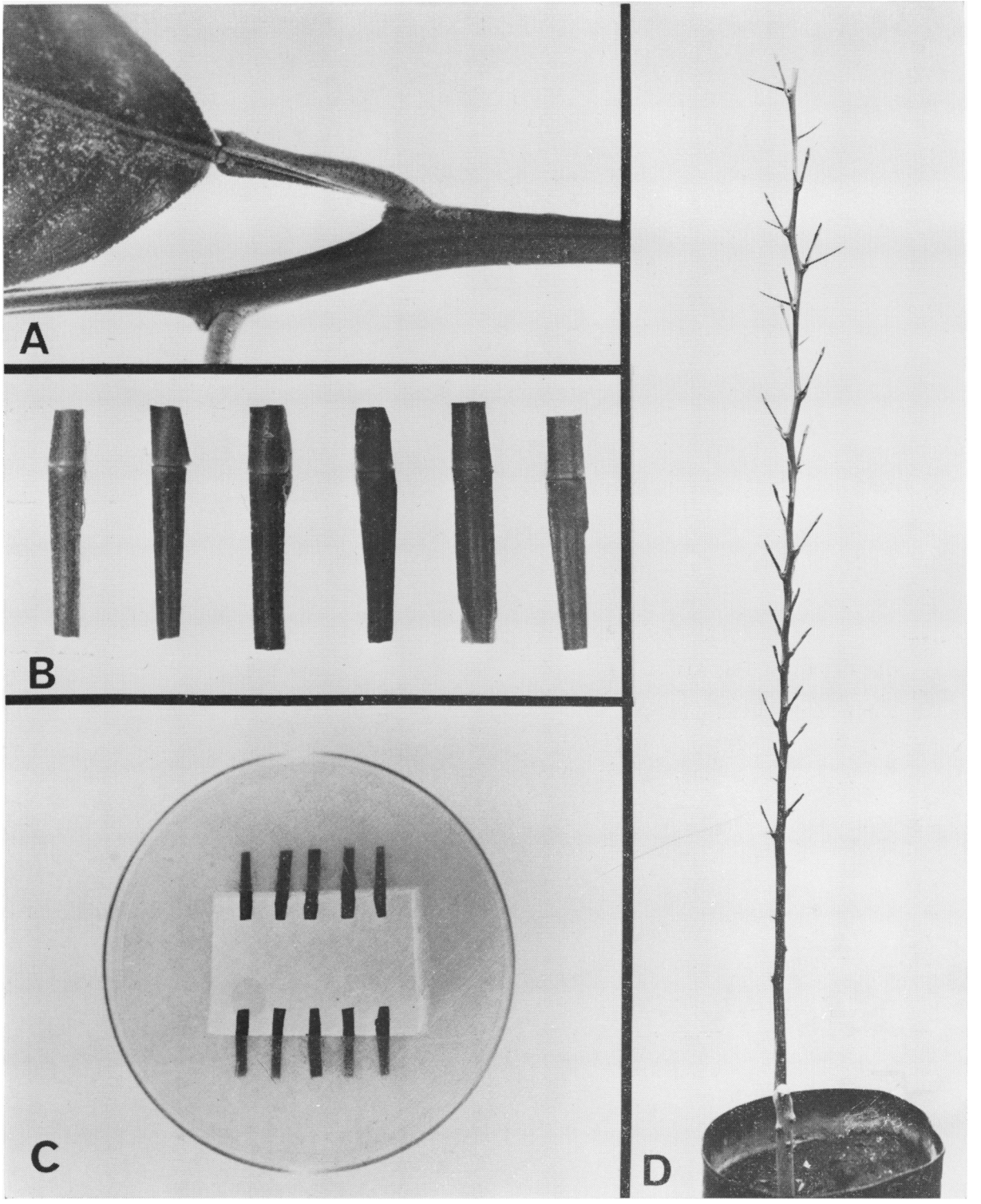

FIG. 1. Citrus leaf explants. A) Intact citrus leaf and petiole. B) Citrus leaf explants. C) Citrus leaf explants mounted in agar. D) Debladed midribs (debladed petioles with 4 mm of leaf midrib remaining).

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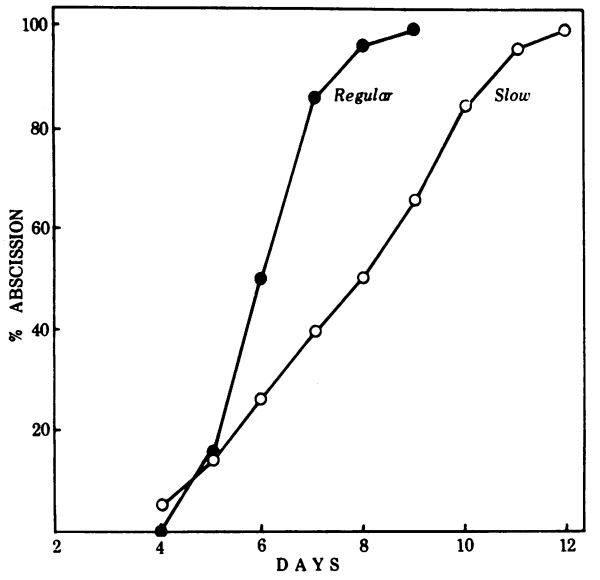
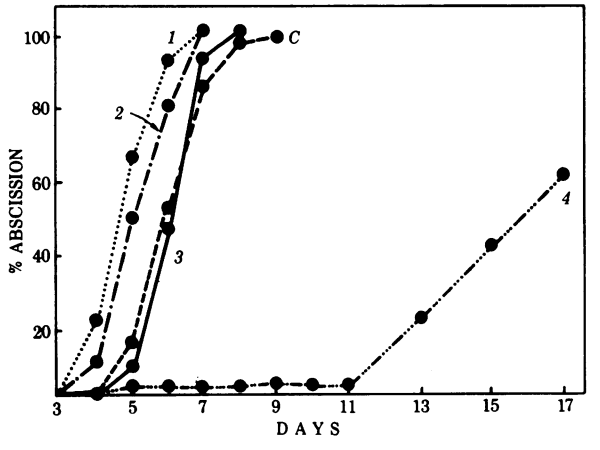




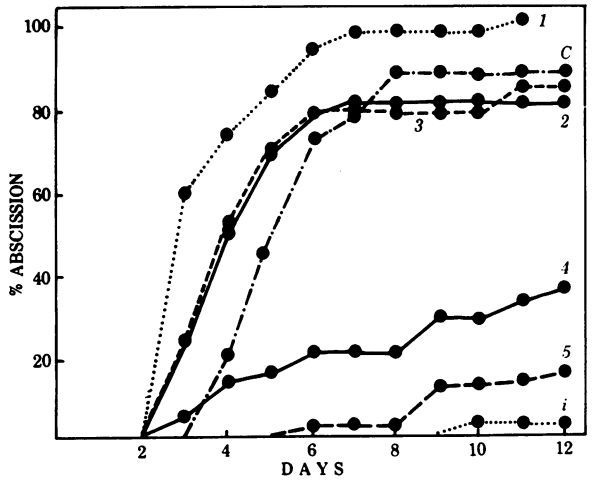


FIG. 3. (Upper) The regular rate of abscission is that observed for control debladed midribs treated with plain agar. The slow rate of abscission results when IAA is applied after deblading to delay abscission for several days, then the IAA is removed and abscission takes place. The time in days for slow abscission was calculated from the time of IAA removal.

Table II. *Response of Debladed Midrib to IAA and GA Applied Alone and Together (Days to 50 % Abscission)*

Times to 50 % abscission were calculated by fitting the data to the straight line equation. Observations were made every 12 hours up to 26 days. Those marked > 26 did not reach 50 % abscission within 26 days after deblading; these values were not used in the statistical analysis. LSD = 0.33 days.

	GA			
IAA	0	10^{-4}	10^{-2}	1
μg/explant		*μg/explant*		
0	5.38	4.65	4.58	4.49
10^{-4}	5.23	5.33	5.14	4.87
10^{-2}	6.70	6.79	6.66	5.34
1	> 26	> 26	> 26	16

debladed midribs (fig 3). This slower rate of abscission was typical of any situation where abscission was delayed for a few days and then continued.

Although the 2 bioassays responded similarly, the debladed midrib had several advantages over the explant: there were no signs of fungal contamination, the results were less variable, the debladed midrib was more like an intact system than the explant, and there was less chance of ethylene being a critical factor, since the debladed midrib was in a ventilated growth chamber while the explant was enclosed in a petri dish. For these reasons, all subsequent experiments were done with the debladed midrib bioassay.

Interaction of GA and IAA Applied to Debladed Midrib Bioassay. This experiment was conducted as a 4 × 4 factorial design with 4 replications of each treatment. GA and IAA were each used at 4 concentrations: 0, 10^{-4}, 10^{-2}, and 1 μg per midrib (table II). When either 10^{-4} or 10^{-2} μg of GA was combined with 1, 10^{-2}, or 10^{-4} μg of IAA, the

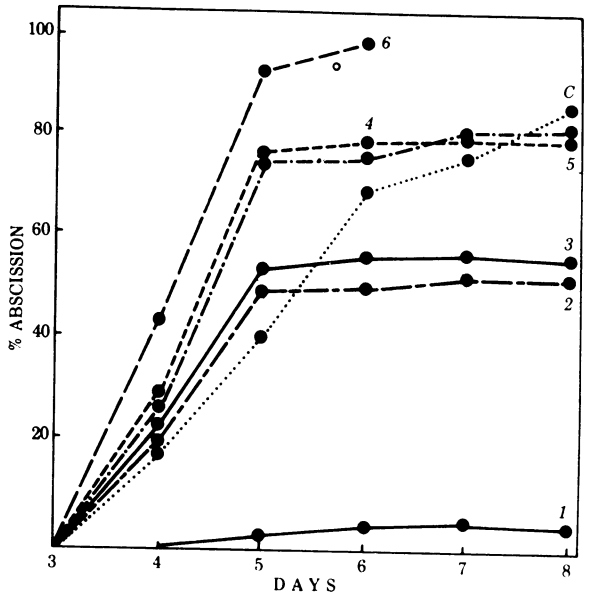
FIG. 4. (Middle) Abscission responses of seedling explants to 1 μg of GA, alone and in combination with various amounts of IAA. The treatments were: 1) 1 μg GA, 2) 1 μg GA + 1 × 10^{-4} μg IAA, 3) 1 μg GA + 1 × 10^{-2} μg IAA, and 4) 1 μg GA + 1 μg IAA; C) Control received plain agar. The calculated time for 50 % abscission for each treatment was: 1) 4.49, 2) 4.87, 3) 5.34, and C) 5.48 days. Treatment 4 was not included in the statistical analysis. LSD at the 5 % level was 0.33 day.

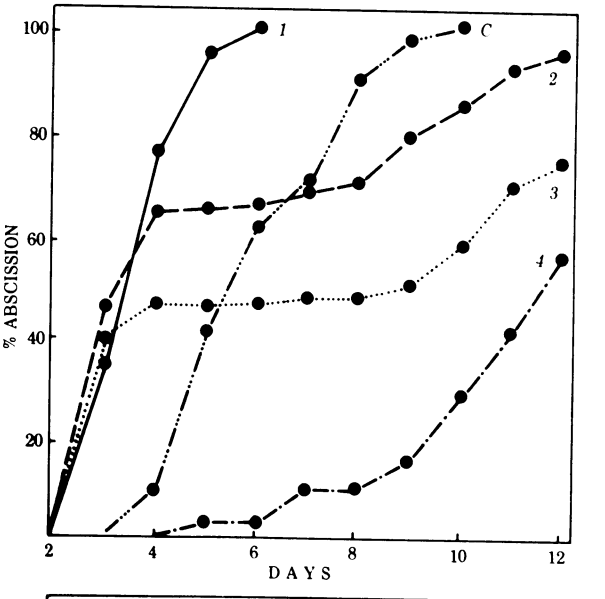
FIG. 5. (Lower) Abscission effects of various amounts of GA applied to each seedling explant with 1 μg of IAA. The treatments were: 1) 32 μg, 2) 16 μg, 3) 8 μg, 4) 4 μg, 5) 2 μg GA, i) IAA alone, and C) Control. LSD at the 5 % level was 22.3, 23.4, 20.7, 10.7, 11.1, 11.1, 10.3, 10.3, 9.1, and 8.4 % from the third to twelfth day of observation, consecutively.

effect of IAA was not altered. That is, at low levels of gibberellin, it was the amount of auxin which regulated abscission. This effect with 10^{-4} μg of IAA was surprising. It was pointed out previously that low levels of auxin seemed to accelerate abscission. Since 10^{-4} and 10^{-2} μg of GA promoted abscission in the absence of IAA, and 10^{-4} μg of IAA tended to increase abscission, it was expected that treating with 10^{-4} μg of IAA + 10^{-4} μg of GA would accelerate abscission, but they did not. Apparently the low concentrations of IAA and GA functioned in an opposing manner, just as did the higher concentrations of the 2 hormones.

Higher levels of GA tended to overcome the abscission delaying effect of IAA (fig 4). One μg of GA accelerated abscission in the presence of 10^{-4} μg IAA, neutralized the retarding effect of 10^{-2} μg of IAA, and shortened the retarding effect of 1 μg of IAA. With 1 μg of GA, 1 μg of IAA retarded abscission until the twelfth day, but then the GA effect became dominant and 50 % of the midribs abscised by the sixteenth day. The length of time that 1 μg of IAA retarded abscission shortened as the concentration of GA increased, until levels of GA were reached which totally overcame the IAA effect. With 2 and 4 μg of GA, IAA retarded abscission for 9 and 3 days, respectively (fig 5). Then abscission proceeded but, like 1 μg GA + 1 μg IAA, at the slower rate than controls. However, 8, 16, or 32 μg of GA + 1 μg IAA significantly decreased the time to 50 % abscission compared to the controls, similar to the effect observed with lower concentrations of GA in the absence of IAA (fig 5). Therefore, the higher concentrations were able to overcome completely the IAA effect.

When 32 μg was added to the open midrib of an intact leaf, 50 % abscission was not reached until 10 days. Apparently, the intact leaf supplied





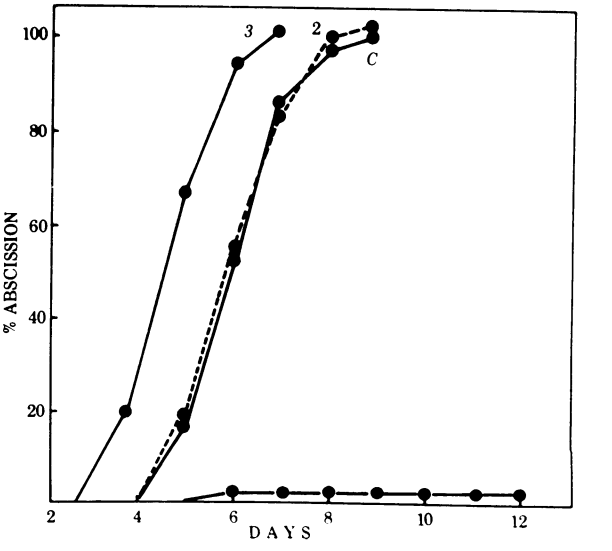


FIG. 6. (Upper) Abscission effects of 1 μg IAA applied to each debladed midrib on seedlings at different time intervals after leaf removal. The time in hours when IAA was applied after leaf removal was: 1) 12, 2) 24, 3) 36, 4) 48, 5) 60, 6) 72, and C) Control (plain agar applied immediately on leaf removal). LSD values were 3.6, 6.7, 8.6, 8.0, and 8.0 % abscission at the 5 % level from fourth to eighth day of observation, consecutively.

FIG. 7. (Middle) Abscission responses to 1 μg IAA applied at different time intervals following a zero hour application of 1 μg of GA to debladed midribs on seedlings. The treatments were: 1) GA applied at zero hour, 2) IAA applied 36 hours after GA, 3) IAA applied 24 hours after GA, 4) IAA applied 12 hours after GA and C) control. LSD at the 5 % level was 10.9, 12.6, 14.8, 10.5, 12.2, 11.9, 19.0, 18.6, and 21.5 % from the third to twelfth day, consecutively.

FIG. 8. (Lower) Abscission responses to GA and IAA in the presence of Barban. The treatments were: 1) IAA or IAA + Barban, 2) GA + Barban, 3) GA, and C) plain agar or plain agar + Barban.

Table III. *Summary of GA/IAA Balance in Abscission*

IAA	GA	Time to 50 % abscission
ug/leaf	*ug/leaf*	*days*
10^{-2}	1	5
1	1	16
1	8	4
1	32	3
Intact leaf	32	10

more auxin to the abscission zone than 1 μg of IAA in agar.

These experiments clearly demonstrate (table III) that the balance of GA and auxin—not the concentration of 1 hormone—is the critical factor in the control of abscission by these 2 hormones.

Two-Stage Effect on Debladed Midrib. When 1 μg of IAA was applied 12 hours after leaf removal, abscission was typically retarded; however, at all later times, the rate of abscission of some leaf midribs was accelerated (fig 6). When the IAA was applied at 24 or 36 hours, about 50 % of the midribs abscised by the fifth day, compared to 40 % of the controls and only 4 % of the 12 hour IAA treated. When the IAA was applied at 48 or 60 hours, about 70 % abscised by the fifth day while the 72 hour application caused 90 % abscission in the same time. There are several significant points about these data which should be noted: A) unlike the explant, there is a 2-stage effect with the debladed midrib; B) the similarity between the 24 and 36 hour and 48 and 60 hour treatments suggests some type of diurnal effect, *i.e.*, the response was evident only at 24 hour points; and C) it appears that the midribs do not all reach stage-2 at the same time—50 % by 24 hours, 70 % by 48 hours, and over 90 % at 72 hours. Those which have not reached stage-2 at the time of IAA treatment can respond to IAA, and they did not abscise for the duration of the experiment. Those which have reached stage-2 abscise more rapidly when treated with IAA.

Since there seemed to be a persistent GA-IAA interaction in these experiments, a study was done to see how delayed applications of IAA would affect the GA response (fig 7). One μg of GA applied alone caused its typical acceleration of abscission, but applications of 1 μg of IAA as late as 36 hours after deblading delayed abscission of some petioles. The 12 hour application of IAA was very similar to the simultaneous application of 2 μg of GA and 1 μg of IAA and there was almost no abscission until about the ninth day. When IAA was applied after 24 hours, 45 % of the sections abscised by the fourth day compared with 10 % for the controls, but there was no further abscission until the ninth day. When IAA was applied after 36 hours, 65 % of the sections abscised by the fourth day and there was no further abscission until the ninth day. In all 3 treatments, abscission proceeded after the ninth day, but at the slower rate (fig 3).

As was observed in figure 6, all of the abscission zones did not become irreversibly engaged in the abscission process at once. Apparently, none are committed to abscise at 12 hours with or without GA, as IAA applied at this time strongly delayed abscission. Since the 24 hour IAA application could not prevent the subsequent abscission of 40 to 50 % of the midribs, it would seem that about one-half of the midribs are irreversibly committed to abscise by 24 hours after deblading. This figure seemed to be the same in the presence or absence of exogenous GA. Even though GA did not increase the rate of commitment to abscise up to the 24 hour point, it did accelerate the rate at which committed midribs completed the abscission process. Without GA, the group committed to abscise during the first 24 hours did so within 5 days, while only 3 days were required with GA. By 36 hours, GA increased the percentage irreversibly committed to abscission to about 65 %, compared with about 50 % in the absence of GA.

The effect of GA on abscission was further characterized by the persistence of the hormone. In the absence of GA abscission of debladed midribs was not resumed, once it was stopped by the delayed application of IAA, but with GA, IAA retarded abscission for about 5 days and then it resumed.

Phenylurethane Effect of Abscission. Because of the anti-gibberellin effect of phenylurethanes recently reported by Yung and Mann (23), Barban and CIPC were tested for their effect on abscission. CIPC had no effect but Barban, which is often more potent than CIPC, eliminated the accelerated abscission normally caused by GA (fig 8). Barban did not affect either the abscission of control tissue or the IAA inhibition of abscission.

Discussion

The interactions of GA and IAA indicate that the balance of the 2 hormones, not the absolute quantity of either is the primary factor in their actions on abscission. The importance of their balance was demonstrated by the increased concentrations of GA required to overcome increasing concentrations of IAA. At low levels of GA, the IAA effect was dominant, but at higher levels of GA, the GA effect controlled abscission. How GA and IAA might compete for control of abscission will not be clear until much more is known about the individual mechanisms of action of plant hormones.

One interaction of GA and IAA which should be discussed is that observed when 1 μg of GA is combined with 1 μg of IAA. In this situation, the IAA delay of abscission persisted for a few days, but then abscission continued at the slower rate

(fig 3) just as if the supply of IAA had been physically removed from the debladed midrib. Perhaps GA has done just that by causing a deterioration of the transport system in the manner suggested for ethylene by Burg and Burg (5). Either the supply of IAA has been cut off from the abscission zone by faulty transport or else the presence of GA has stimulated the accumulation of some factor that causes the cells to act as if there were no IAA there, and abscission follows. It would seem reasonable to expect that this unknown factor might be an enzyme or group of enzymes since the role of gibberellin as a stimulator of enzyme synthesis is well documented (7, 21).

Certain segments of the responses seen in abscission can be explained by mechanisms of nucleic acid synthesis. Results of several workers (9, 12, 16) indicate that the action of IAA depends on continued synthesis of mRNA because of a 2 or 3 hour half-life of the template material, but that IAA does not de-repress a genome for synthesis of a mRNA containing new information. If the argument is presented that IAA represses synthesis of abscission inducing mRNA, then GA or just the absence of IAA (*c.g.* the controls) must de-repress this system to induce abscission. But Barban blocked only the GA accelerating action not the abscission of central midribs. This explanation needs a de-repression system independent of GA and one dependent on GA. This may be possible but it seems cumbersome.

Finally, other workers have clearly demonstrated that protein synthesis is essential for IAA action (15), and Winter and Thimann (21) reported that IAA was bound to the protein fraction in coleoptile sections. Sarkissian and Daniel (20) have also suggested an allosteric protein-IAA mechanism for the very rapid response observed in the IAA stimulation of oxidative phosphorylation. Recently, Sarkissian (19) reported an *in vitro* system where IAA reduced the number of protein bands observable on an electrophoretic plate. He suggested this may result from an allosteric protein-IAA effect. Such an IAA-protein complex could provide the enzymatic control of abscission if the protein accelerated abscission in the absence of IAA and retarded abscission in its presence.

Acknowledgment

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